Genetics of Adolescent/Young Adult ALL (Cytogenetics)

Christine J Harrison
Professor of Childhood Cancer Cytogenetics
Cytogenetic subgroup by age

Moorman 2007
AYA by Age at Diagnosis and Treatment Trial
1990-present (n=1,205)

- ALL2003 (N=384)
- ALL97 (N=221)
- UKALLXI (N=108)
- UKALLXII (N=492)
Adolescents With Acute Lymphoblastic Leukaemia: Outcome on UK National Paediatric (ALL97) and Adult (UKALLXII/E2993) Trials

Ramya Ramanujachar, MRCPCH, Sue Richards, PhD, Ian Hann, MD, Anthony Goldstone, MD, Christopher Mitchell, PhD, Ajay Vora, MD, Jacob Rowe, MD, and David Webb, MD

Adolescents With Acute Lymphoblastic Leukaemia

Fig. 1. Overall survival of patients aged 15, 16 and 17 years in the UKALL trials; Abbreviations used: Obs, observed, Exp, expected.
Fig. 2. Event free survival of patients aged 15, 16 and 17 years in the UKALL trials; Abbreviations used: Obs, observed; Exp, expected.
Age groups (n=1,205)

- 15-19 years: 544, 45%
- 13-14 years: 432, 36%
- 20-24 years: 229, 19%
Sex Ratio (1.74M:1F)

Males, 765, 63%

Females, 440, 37%
Immunophenotype (n=1,132)

- BCP-ALL, N=894, 79%
- T-ALL, N=234, 21%
- Mature-B, N=4, 0%

Immunophenotype not known in 73 (6%) cases
Age-specific incidence of T-ALL
Estimates of the incidence of T-ALL specific abnormalities

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children</td>
</tr>
<tr>
<td>SIL-TAL1/t(1;14)</td>
<td>22%</td>
</tr>
<tr>
<td>t(11;14)(p13;q11)/LMO2</td>
<td>12%</td>
</tr>
<tr>
<td>t(10;14)/TLX1 (HOX11)</td>
<td>2%</td>
</tr>
<tr>
<td>t(5;14)/TLX3 (HOX11L2)</td>
<td>17%</td>
</tr>
<tr>
<td>CALM-AF10</td>
<td>2%</td>
</tr>
<tr>
<td>CDKN2A/B</td>
<td>51%</td>
</tr>
<tr>
<td>MLL</td>
<td>4%</td>
</tr>
<tr>
<td>NUP214-ABL1</td>
<td>2%</td>
</tr>
</tbody>
</table>
Cytogenetics of BCP-ALL in 13-24 year olds (n=837)

- Normal: 13%
- Other: 27%
- IGH@-CRLF2: 3%
- IGH@: 12%
- CRLF2: 3%
- iAMP21: 4%
- HeH: 16%
- Hypo (<40): 3%
- t(1;19): 3%
- t(4;11): 3%
- t(12;21): 4%
- t(17;19): 0%
- t(17;21): 0%
- t(17;19): 0%
- 11q23: 1%
- t(17;19): 0%
Estimates of the incidence of BCP-ALL specific abnormalities

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>No. Positive</th>
<th>No. Tested</th>
<th>Incidence</th>
<th>&lt;13 years</th>
<th>&gt;24 years</th>
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</thead>
<tbody>
<tr>
<td>t(9;22)</td>
<td>68</td>
<td>781</td>
<td>9%</td>
<td>2%</td>
<td>20%</td>
</tr>
<tr>
<td>t(1;19)</td>
<td>27</td>
<td>696</td>
<td>4%</td>
<td>3-5%</td>
<td>3-5%</td>
</tr>
<tr>
<td>t(12;21)</td>
<td>25</td>
<td>531</td>
<td>5%</td>
<td>25%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(17;19)</td>
<td>4</td>
<td>696</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(4;11)</td>
<td>27</td>
<td>780</td>
<td>4%</td>
<td>2%</td>
<td>5-10%</td>
</tr>
<tr>
<td>11q23</td>
<td>6</td>
<td>780</td>
<td>1%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>HeH</td>
<td>149</td>
<td>754</td>
<td>20%</td>
<td>35%</td>
<td>10%</td>
</tr>
<tr>
<td>Hypo (&lt;40)</td>
<td>23</td>
<td>754</td>
<td>3%</td>
<td>1%</td>
<td>5%</td>
</tr>
<tr>
<td>iAMP21</td>
<td>26</td>
<td>531</td>
<td>5%</td>
<td>&lt;2%</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>IGH@</td>
<td>31</td>
<td>216</td>
<td>14%</td>
<td>3%</td>
<td>15%</td>
</tr>
<tr>
<td>IGH@-CRLF2</td>
<td>8</td>
<td>284</td>
<td>3%</td>
<td>&lt;1%</td>
<td>~5%</td>
</tr>
<tr>
<td>CRLF2</td>
<td>5</td>
<td>115</td>
<td>4%</td>
<td>~5%</td>
<td>?</td>
</tr>
<tr>
<td>Normal</td>
<td>102</td>
<td>696</td>
<td>15%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>227</td>
<td>696</td>
<td>33%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4 cases had iAMP21 plus CRLF2 and 2 cases had iAMP21 plus an IGH translocation
“Others”

Total n=227

- Abnormal 9p ~50%
- +21 ~4%
- +8 ~4%
- +5 ~4%
Short communication

Is trisomy 5 a distinct cytogenetic subgroup in acute lymphoblastic leukemia?

Rachel L. Harris, Christine J. Harrison, Mary Martineau, Kerry E. Taylor, Anthony V. Moorman*

Table 1
Clinical, survival, cytogenetic and FISH data for seven patients with ALL and trisomy 5

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (yr)/Sex</th>
<th>Diagnosis</th>
<th>WBC ($\times 10^9$/L)</th>
<th>1st Rel (mo)</th>
<th>2nd Rel (mo)</th>
<th>Overall survival (mo)</th>
<th>Karyotype</th>
<th>interphase FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3112</td>
<td>7/M</td>
<td>Com/pre-B ALL</td>
<td>88.0</td>
<td>43</td>
<td>—</td>
<td>55+</td>
<td>47,XY,+5[9]/46,XY[1].ish +5(wcp5+)</td>
<td>Neg</td>
</tr>
<tr>
<td>1642</td>
<td>9/M</td>
<td>Com ALL</td>
<td>13.3</td>
<td>—</td>
<td>—</td>
<td>82+</td>
<td>47,XY,+5[5]/46,XY[3].ish +5(wcp5+)</td>
<td>—</td>
</tr>
<tr>
<td>2955</td>
<td>10/M</td>
<td>Com/pre-B ALL</td>
<td>5.3</td>
<td>33</td>
<td>—</td>
<td>33+</td>
<td>47,XY,+5[20]</td>
<td>Neg</td>
</tr>
<tr>
<td>1323</td>
<td>14/M</td>
<td>Com ALL</td>
<td>19.0</td>
<td>38</td>
<td>50</td>
<td>52</td>
<td>47,XY,+5[6]/46,XY[4]</td>
<td>—</td>
</tr>
<tr>
<td>3209</td>
<td>14/M</td>
<td>Com/pre-B ALL</td>
<td>1.4</td>
<td>—</td>
<td>—</td>
<td>53+</td>
<td>46,X,-Y,+5[6]/46,XY[8]</td>
<td>Neg</td>
</tr>
<tr>
<td>4765</td>
<td>27/M</td>
<td>Pre-B ALL</td>
<td>17.6</td>
<td>—</td>
<td>—</td>
<td>14+</td>
<td>47,XY,+5[6]/46,XY[7]</td>
<td>—</td>
</tr>
<tr>
<td>2478</td>
<td>31/F</td>
<td>Com ALL</td>
<td>7.4</td>
<td>37</td>
<td>41</td>
<td>43</td>
<td>47,XX,+5[3]/46,XX,+8[4]/46,XX[2]</td>
<td>—</td>
</tr>
</tbody>
</table>

The common/pre-B immunophenotype was CD10+, CD19+; cytoplasmic μ-chain was not tested.

*Abbreviations: Com, common; Neg, negative; Rel, relapse; WBC, white blood cell count.
Duplication of chromosome 21 involving amplification of RUNX1

Intrachromosomal amplification of chromosome 21 iAMP21
Amplification of *AML1* on a duplicated chromosome 21 in acute lymphoblastic leukemia: a study of 20 cases

L Harewood¹,², H Robinson¹, R Harris¹, M Jabbar Al-Obaidi¹, GR Jalali¹, M Martineau¹, AV Moorman¹, N Sumption¹, S Richards², C Mitchell¹ and CJ Harrison¹ on behalf of the Medical Research Council Childhood and Adult Leukaemia Working Parties

¹Leukaemia Research Fund Cytogenetics Group, Cancer Sciences Division, University of Southampton, Southampton, UK; ²Clinical Trial Service Unit, Radcliffe Infirmary, Oxford, UK; and ³Paediatric Oncology, John Radcliffe Hospital, Oxford, UK
Amplification of band q22 of chromosome 21, including AML1, in older children with acute lymphoblastic leukemia: an emerging molecular cytogenetic subgroup

Leukemia (2003) 17, 1679–1682. doi:10.1038/sj.leu.2403000

TO THE EDITOR

J Soullet
L Trakhtenbrot
V Najfeld
JM Lipton
S Mathew
H Avet-Loiseau
M De Braekeleer
S Salem
A Baruchel
SC Raimondi
SD Raynaud

1 Centre Hospitalier Universitaire (CHU) Saint Louis, AP-HP, Paris, France;
2 The Chaim Sheba Medical Center, Tel-Hashomer, Israel;
3 The Mount Sinai Medical Center, New York, NY, USA;
4 New York Presbyterian Hospital-Cornell Campus Cornell University Weill Medical College, New York, NY, USA;
5 CHU Nantes, France;
6 CHU Brest, France;
7 CHU Nice, France;
8 Jude Children’s Research Hospital, Memphis, TN, USA
Demographic Profile of iAMP21 patients

- Older children/Adolescents
  - Median age 10 years
- Common/Pre-B immunophenotype
- Low WBC
EFS of 28 iAMP21 patients on MRC ALL97

Outcome of iAMP21 patients on MRC ALL97

- Overall Survival - 5yr 69% (SE 8.6)
- Event Free Survival - 5yr 26% (SE 8.3)

N=29
22 relapses (15 BM, 5 CNS, 2 BM&CNS)
11 deaths (1 in 1st CCR)

October 2007 Update
Decision

To treat iAMP21 patients as high-risk in the current childhood trial: ALL2003
iAMP21: outcome in ALL2003

- Two deaths in remission
- Infection following BMT
- One BM relapse
- Post BMT
- 9yr, Female

n=39
33 treated as high risk
14 received BMT
iAMP21
Duplication of chromosome 21 involving amplification of RUNX1

Every abnormal chromosome 21 has a different morphology
Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21

Jon C. Strefford*, Frederik W. van Delft†, Hazel M. Robinson*, Helen Worley*, Olga Yiannikouris†,
Rebecca Selzer†, Todd Richmond†, Ian Hann**, Tony Bellotti†, Manoj Raghavan†, Bryan D. Young†,
Vaskar Saha†, and Christine J. Harrison**

*Leukaemia Research Cytogenetics Group, Cancer Sciences Division, University of Southampton, Southampton S016 6YD, United Kingdom; †Cancer Research UK Children’s Carfia Group and Medical Oncology Unit, Institute of Cancer, Queen Mary University of London, London E1 4NS, United Kingdom;
**Department of Haematology, Great Ormond Street Hospital for Children NHS Trust, London WC1N 3JH, United Kingdom; and ††Computer Learning Research Centre, Royal Holloway, University of London, Egham, Surrey TW20 0EX, United Kingdom
A double-strand DNA break results in loss of a telomere and the formation of an unstable chromosome.

Following replication the two sister chromatids fuse to form a dicentric chromosome.

During anaphase this dicentric pulls apart resulting in breakage of the fusion bridge and production of an unstable chromosome with an inverted repeat.

This process is repeated until the chromosome becomes stabilised by gaining a telomere. In this way it is possible to generate a chromosome with ladder-like amplification.
iAMP21

• iAMP21 defines a distinct patient subgroup of older children/young adults with a poor prognosis
• Chromosomal instability gives rise to complex intrachromosomal rearrangements of chromosome 21
• Genome wide they show the same abnormalities of B-cell differentiation genes
• No obvious differentially expressed genes
• Studies are in progress to determine the initiating mechanism
• Currently FISH with probes directed to \textit{RUNX1} is the only reliable diagnostic method
**IGH@ translocations in BCP-ALL**

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**BRIEF COMMUNICATION**

**t(14;19)(q32;q13): A Recurrent Translocation in B-Cell Precursor Acute Lymphoblastic Leukemia**

Hazel M. Robinson, Kerry E. Taylor, G. Reza Jalali, Kan Luk Cheung, Christine J. Harrison, and Anthony V. Moorman*

Leukaemia Research Fund Cytogenetics Group, Cancer Sciences Division, University of Southampton, Southampton, UK.
Overexpression of CEBPA resulting from the translocation t(14;19)(q32;q13) of human precursor B acute lymphoblastic leukemia

Within 6 nucleotides

IGH@-CEBP family
Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL)

IGH Testing in ALL by Age (n=1,304)
3% <10 yrs, 14% >10 years
NB Selected screening

![Bar chart showing the number of patients in different age groups with normal and translocation results.](attachment:image.png)
IGH@-CEBP family

t(14;14)(q11;q32)  
CEBPE  
der(14)  
der(14)

t(14;20)(q32;q13)  
CEBPB  
der(14)  
der(20)

t(14;19)(q32;q13)  
CEBPA  
der(14)  
der(19)

t(8;14)(q11;q32)  
CEBPD  
der(8)  
der(14)

IGH@ translocations
IGH@-CEBPG
IGH@-CEBP family

t(14;14)(q11;q32) inv(14)(q11q32)

t(14;19)(q32;q13)

t(14;20)(q32;q13)

t(8;14)(q11;q32)
$\text{IGH}^{\#}$-CEBP family

qRTPCR

Fold Change

$\text{t}(8;14)$  $\text{t}(8;14)$  $\text{t}(4;14)$  HL60  MUTZ5  REH  SD1  TANOUUE  PER365  ALL patient (C)  ALL patient (M)  BM

*CEBPB*  *CEBPD*

Samples
### IGH@-CEBP family

<table>
<thead>
<tr>
<th>Translocation</th>
<th>M:F ratio</th>
<th>Age range (median)</th>
<th>WBC range x10⁹/L (median)</th>
<th>Current status where available</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(14;19)(q32;q13)</td>
<td>2:7</td>
<td>10-44 (19)</td>
<td>1-71 (5)</td>
<td>1 dead 4 CR</td>
</tr>
<tr>
<td>t(14;19)(q32;q13)</td>
<td>0:1</td>
<td>32</td>
<td>94</td>
<td>NA</td>
</tr>
<tr>
<td>t(14;20)(q32;q13)</td>
<td>1:2</td>
<td>13-35 (15)</td>
<td>3-103 (75)</td>
<td>2 CR</td>
</tr>
<tr>
<td>t(8;14)(q11;q32)</td>
<td>5:5</td>
<td>3-49 (14)</td>
<td>2-375 (7)</td>
<td>1 dead 2 CR</td>
</tr>
<tr>
<td>t(14;14)(q11;q32)</td>
<td>4:0</td>
<td>15-45 (20)</td>
<td>1-24 (13)</td>
<td>3 CR</td>
</tr>
<tr>
<td>inv(14)(q11q32)</td>
<td></td>
<td></td>
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</tbody>
</table>
Summary – *IGH@*-CEBP family

- Four *IGH@* translocations
- Involve five partner genes from the same gene family – CCAAT enhancer binding-proteins
- One subtype of haematological disease, B-cell precursor ALL in older children and young adults
- Basic leucine zipper transcription factors implicated in proliferation and differentiation
- Expressed in haematopoietic system – control of myeloid differentiation
- Tumour suppressor and oncogenic effects in leukaemogenesis
t(6;14)(p22;q32): a new recurrent *IGH*@ translocation involving *ID4* in B-cell precursor acute lymphoblastic leukemia (BCP-ALL)

Lisa J. Russell,¹ Takashi Akasaka,² Aneesha Majid,² Kei-ji Sugimoto,² E. Loraine Karran,² Inga Nagel,³ Lena Harder,³ Alexander Claviez,⁴ Stefan Gesk,² Anthony V. Moorman,¹ Fiona Ross,⁵ Helen Mazzullo,⁶ Jonathan C. Strøfford,¹ Reiner Siebert,³ Martin J. S. Dyer,² and Christine J. Harrison¹

¹Leukaemia Research Cytogenetics Group, Cancer Sciences Division, University of Southampton, Southampton General Hospital, Southampton, United Kingdom; ²Medical Research Council (MRC) Toxicology Unit, University of Leicester, Leicester, United Kingdom; ³Institute of Human Genetics and ⁴Department of Pediatrics, University-Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; ⁵Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, United Kingdom; and ⁶Department of Haematology and Blood Transfusion, University Hospital, London, United Kingdom
IGH@-ID4

bHLH family of transcription factors – inhibitory proteins which regulate growth, differentiation, senescence and apoptosis
**IGH@-ID4**

- qRTPCR

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**Fold change**

<table>
<thead>
<tr>
<th>Samples</th>
<th>6120</th>
<th>16503</th>
<th>Normal Thyroid</th>
<th>BCP-ALL patient without t(6;14)</th>
<th>BCP-ALL patient without t(6;14)</th>
<th>Normal bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ID4</strong></td>
<td><img src="image" alt="ID4" /></td>
<td><img src="image" alt="ID4" /></td>
<td><img src="image" alt="ID4" /></td>
<td><img src="image" alt="ID4" /></td>
<td><img src="image" alt="ID4" /></td>
<td><img src="image" alt="ID4" /></td>
</tr>
</tbody>
</table>
IGH@-ID4

Patients

- 13 BCP-ALL patients – recurrent translocation
- Low WBC (median $3 \times 10^9/l$, range 1-11$x10^9/l$)
- Age higher than expected for BCP-ALL (median 16 yrs, range 6-48 years)
LETTER TO THE EDITOR

A novel translocation, t(14;19)(q32;p13), involving IGH@ and the cytokine receptor for erythropoietin

---

**Leukemia (2008), 1-4**
© 2008 Macmillan Publishers Limited. All rights reserved 0887-6924/08 $32.00
www.nature.com/leu
IGH@-EPOR

- qRTPCR
IGH@-CRLF2

TSLP (thymic stromal derived lymphopoietin)
IGH@-CRLF2

- 33 patients

- BCP-ALL
  - CD34+ and CD33+
  - Median age 16yrs (range 3-76yrs)

- t(X;14)(p22;q32)
- t(Y;14)(p11;q32)
- Unknown
11 patients

27 BCP-ALL cell lines – 2 with t(Y;14)
IGH@-CRLF2

- Expression

patients with trans

patients without trans

cell lines with trans

cell lines without trans

normal bone marrow

p<0.0001

Delta Ct values

Events

10^0 10^1 10^2 10^3 10^4

PE-A
Children (n=19)
10 events: 8 relapses (7 died); 2 non-remitter/early death
9 patients on ALL2003 – all in 1st CCR
IGH@ Partners

Published by LRCG and collaborators

Previously reported

On-going

IGH@ Translocations
IGH@ Partners in BCP-ALL

Previously reported
Published by LRCG and collaborators
On-going

BCP-ALL

IGH@

Translocations

1q24 - LHX4
1q21 - BCL9
1q24 - LHX4
6p22 - ID4*
7p14 – TRG@
8q11 - CEBPD
5q31 - IL3
14q11 - CEBPE
12p13 – BCL1

19q13 - CEBPA
19q13 - CEBPG
19p13 - EPOR
20q13 - CEBPB

Xp22/Yp11 – CRLF2
IGH@ translocations

• IGH@ is a promiscuous locus: common link to the genes involved and their interrelated pathways
• Majority of patients are older children or adolescents
• Cytogenetics still identifies new translocations and subgroups
Conclusions to genetics of AYA

• They show abnormalities in common with childhood ALL, although the incidences are different

• There are some novel abnormalities emerging which are common in this age group

• Detailed analysis may highlight some as these as specific targets for therapy
Acknowledgements

Newcastle, UK
- Anthony Moorman
- Leukaemia Research Cytogenetics Group

Leicester, UK
- Martin Dyer

Kiel, Germany
- Reiner Siebert

Paris, France
- Olivier Bernard
To find *IGH@* positive cases

Screen by FISH with *IGH@* breakapart probe

Not:

- *ETV6-RUNX1* positive
- High hyperdiploidy
- *BCR-ABL1*
- *t(1;19)*
IGH@-CRLF2

- aCGH
IGH@-CRLF2

• Knockdown

[Graph showing fold change for siCRLF2 and Negative control siRNA]

Day 3

<table>
<thead>
<tr>
<th></th>
<th>60KDa-</th>
<th>43KDa-</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS 071 195</td>
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<td></td>
</tr>
</tbody>
</table>

Day 4

<table>
<thead>
<tr>
<th></th>
<th>CRLF2</th>
<th>β-Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS 071 195</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Bar chart showing events for different conditions]
IGH@-CRLF2

- Biological consequences

No difference in;

- Apoptosis
- pSTAT5
- Cell cycle

Why?
IGH@-CRLF2

- JAK2 mutation?
  - WT: TTCTGCTTATCAGAGAAGAA
  - GGA: R683G
  - TTC: I682F
**IGH@-CRLF2**

- Retroviral transfection

Mouse fetal liver cells

![Graph showing number of colonies over plating](image)

hCRLF2 – ↑number and ↑diameter

![Images of CRLF2 and EV](image)

Data from Dr Melania Capasso
**IGH@-CRLF2**

CD43+/CD19+

- CRLF2 expressing cells are less differentiated compared to EV cells
- Low CRLF2 expressing cells are more differentiated than high CRLF2 expressing cells

Data from Dr Melania Capasso
Numbers of AYA by Trial and Year of diagnosis (N=1,179)

- ALL2003 (N=335)
- ALL97 (N=243)
- UKALLXI (N=107)
- UKALLXII (N=493)